Cal Poly Dairy Embryo Recovery Record System

By

Kelsey Donahoo-Cheda

Dairy Science Department

College of Agriculture

CALIFORNIA POLYTECHNIC STATE UNIVERSITY

San Luis Obispo

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ABSTRACT

The objective of this paper was to generate a record system for the Embryo Collection and Transfer, which is efficient and accurate for the California Polytechnic State University Dairy. By creating this system, less confusion and more organized documentations will be placed in effect in the conclusion of this piece. This will help unify the system, taking into account a high turnover rate with student employees. By creating an easy to understand and usable system it will be simple to maintain accurate records. After close work with Dr. Stan Henderson, Mr. Rich Silacci and Ms. Daniela Demerio, records were obtained of all Cal Poly dairy cattle including: breed registration, pedigree, DNA sample, inventory of all flushes, recipients, frozen and fresh implants, pregnancies, births, sales, and embryo transfer and recovery records. Starting as early as 2005 data were collected involving transfers, frozen inventory, individual cow identification and work related to the list above. Costs and economic value was also evaluated. Results are a binder with the data collected on each individual. The successful Cal Poly Classic Sale 2010 was a realist method of marketing Cal Poly embryo transfers and embryos and used the record system to ensure the right paperwork was filed and correct animal was sold. When provided with a true system of records, efficiency and accuracy increases, and confusion decrease.

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INTRODUCTION

Embryo Transfer (E.T.) has been a popular method to increase progeny from extraordinary female cattle for decades. The Cal Poly Dairy is no exception in following this trend and for a several years has been developing an organized E.T. program. Over the past couple of years, Cal Poly Dairy has worked with embryology experts to choose females then flush and acquire embryos from these cattle in the Holstein and Jersey herds. With many embryos now flushed and stored, an accurate and efficient record system is necessary to help decrease confusion and provide an easy manageable way to understand the Cal Poly Dairy embryo program.

With a large number of student employees, there is a large turnover rate of employees at the Cal Poly Dairy including herdsmen and breeders. It is vital that a system is created to keep confusion minimal when changes are made in employment. This will be done by furthering the program to include assisting in breed association paperwork, maintaining an accurate record of fresh implant embryos, DNA sampling, recipients of embryos, registration forms and records of births, flushes and frozen embryos. This program may also benefit the dairy in difficult economic times. Since the program has started, Cal Poly's embryos have been marketed and used the enclosed record system to be sure the proper paperwork was completed and proper embryos and calves were sold. The author has worked very closely with Dr. Stan Henderson, herd manager Rich Salicci and embryologist Daniela Demetrio to progress the current development of the record system. This paper analyzes relevant research in relationship to Embryo Transfer and associating processes, outlines materials used, and illustrates the current and future benefits.

LITERATURE REVIEW

Choosing A Donor

The collection process begins with a donor cow. This is a cow that has been selected from a herd because she excels in traits needed by perspective buyers. For each dairyman these traits might be different. For example, she might be high in milk production, fat, protein and so on or she could be the dairy strong, overall correct appearing cow. Whatever the motivation may be for choosing her to conduct an embryo transfer on, will have based on potential embryo market demand.

Basic Vocabulary

According to Dr. J.R. Kunkel from University of Vermont (*Kunkel, J.R.. 2010*), Embryo Transfer is defined as the process by which an embryo is collected (flushed) from one female (the donor) and transferred to another female (the recipient) to complete the gestation period. Wikipedia (2010) defines Embryo Transfer, referring to a step in the process of in-vitro fertilization (IVF) whereby one or several embryos are placed into the uterus of the female with the intent to establish a pregnancy. Fundamentally, a fertilized egg is transferred from the biological mother to a recipient that will then carry out the rest of the gestation of that embryo. Embryo can be defined as a fertilized ovum that will eventually develop into the offspring (Kunkel, J.R.. 2010). Before the transfer work can be completed the donor cow will go through an ovulation stimulating process using a follicle stimulating hormone, FSH, to produce more than one ovum. This is referred to as Superovulation. Another process that can be completed for a fresh transfer would be the synchronization of estrous cycles between the donor and the recipient. Injections of prostagladin will help stimulate the onset of estrus or heat.

Achievement of ET work

The whole process seems confusing at first but with detailed and accurate information it can be explained easily. First, what is the purpose of doing embryo transfer (ET) procedure? It is thought that with the development of ET that overpopulation would become a huge problem. Although numbers have increased, an extreme overpopulation has not fully developed. The production of offspring has increased since first brought to dairy cattle but mostly for genetic superiority and research of the animal. This experiment has allowed the dairy industry to increase numbers of genetically superior cows, number of offspring in a lifetime, disease control and the genetic change within small populations. It has also helped decrease variability in research subjects for the studies of physiology, pathology, immunology of reproduction and more. We now have higher producing cows with longer life spans because of the strong genetics behind each cow, which ET helped achieve. The whole embryo transfer process from start to finish takes up to 21 Days. After a donor is selected she is observed to record her estrous cycles. There are two different options from here.

Superovulation

If a single ovum is desired, 6-8 days after her normal estrous and breeding she can be flushed. In relationship to a fresh transfer the recipient will have to be in estrus at the same time to accept the embryo to complete gestation. Usually, synchronizing the estrus in the recipient is done at the same time as the donor.

Second, if multiple ova are preferred then superovulation will need to occur. This is when the donor cow is treated with gonadotropin, a follicle simulating hormone (FSH). She receives FSH between day 9 and 14 of her estrous cycle where day 0 equals estrus.

FSH is given twice a day for 4 to 5 days. This produces an outcome of multiple follicles on the ovaries in the donor.

Prostaglandin injections are given to both the donor and the recipient 2 or 3 days after beginning FSH injections. This will help start the synchronized estrous stage between the donor and recipient in 2 to 3 days after the first injection.

Insemination

When the donor cow comes into estrus (standing heat) after the prostaglandin injections, semen will then be deposited in the body of the uterus. The use of frozen semen straws are the usual choice due to the fact that genetics are more assessable and chosen for trait improvement. Up to 3 inseminations are recommended at 12, 24 and 36 hours after the start of estrus. Since ET requires both time and effort and the person doing the transfer wants good results, higher quality semen and sexed semen are usually used to increase the odds of a health female offspring. The embryos are then flushed from the donor 6 to 8 days after insemination.

Collection of Embryos/ Flushing

The actual process of flushing is not complicated in itself. The basic anatomy of a dairy cow's reproductive tract does not differ substantially from the human female. The basic structures in respective order from most outer layer to inner organ are as follows: vagina, cervix, uterus, ovary. Of the major structures the major difference in what connects the ovary to the uterus. For a human it is a fallopian tube, for cattle it is an oviduct. When it is time to extract the embryos from the donor cow, or flushing, a flexible catheter is inserted first with a stiff probe.

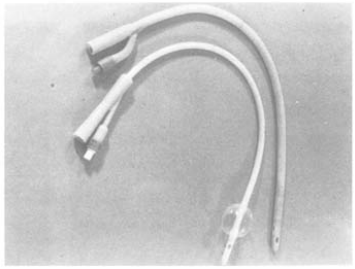


Figure 1: Catheter (Seidel, G.E.et al, 1991)

The catheter has an opening in which the embryos are guided through and out of the cow. The catheter is held in place by a balloon like feature. This balloon has a separate chamber down the catheter so that at the opposite end a syringe can be inserted and blow the balloon like feature up with air. This probe helps guide the catheter through the cervix, which unlike humans, has a folded pathway through it. By rectal palpation, maneuvering the cervix around the probe and will be guided through to the other side of the cervix, coming out into the uterine body.

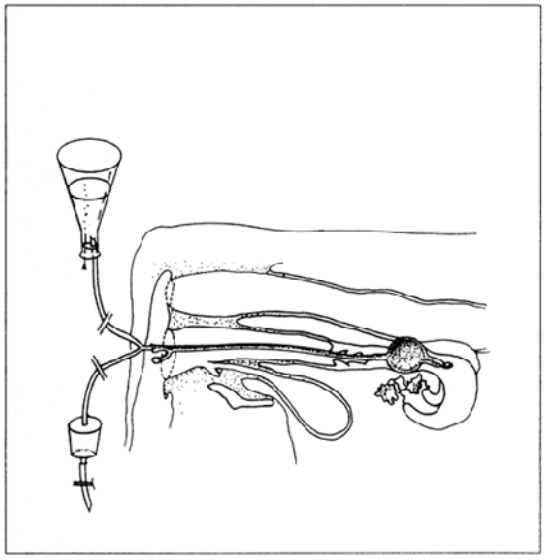


Figure 2: Non-Surgical Embryo Recovery (Seidel, G.E.et al, 1991)

Once into the uterine body there are two different ways to flush out the uterine horns. The first way is the traditional way, first used by many veterinarians and ET

specialists. After the catheter is passed the cervix the balloon could be filled with air and block anything from exiting the cervix. The newer most recent technique is by entering the catheter in one uterine horn at a time. Perhaps, entering the right uterine horn, filling the balloon with air and blocking anything from exiting the right uterine horn, flushing the embryo(s) then moving to the left.

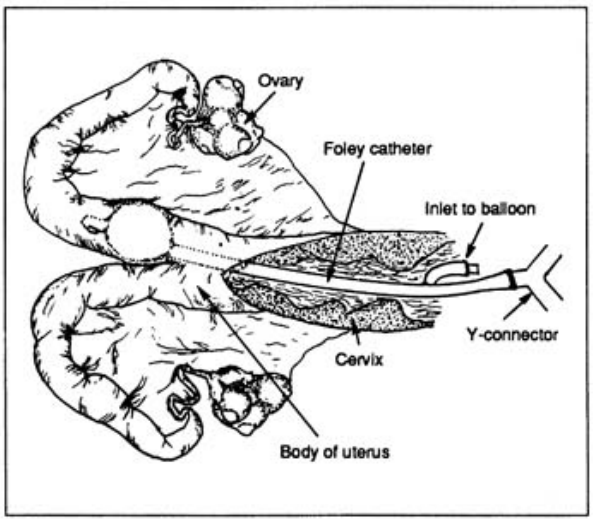


Figure 3: Horn Flushing (Seidel, G.E. et al, 1991)

Once the balloon is full of air blocking whichever exit is decided on, the probe is removed. At the end of the catheter that is outside of the cow there are three different tubes. One which was discussed above was the air tube to fill the balloon feature. The other two are for the saline solution or other suitable culture media. One tube going in, the other coming out. The saline solution is usually a continuous flow or intermittent flushed of 30-200 milliliters. When the flow in has stopped, the out flow begins. The saline solution has picked up the embryos within the donor, so when the outward flow begins they are carried out. At the end of the exit tube is an embryo filter, which contains

an extremely small screen at the bottom. This allows excess fluid to run out and the embryos stay caught on the screen. If this was done in one horn then it would be repeated for the opposite horn.

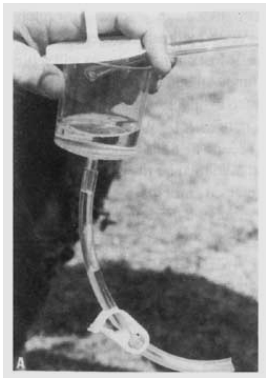


Figure 4: Collection Tube and Filter (Seidel, G.E. et al, 1991)

Evaluation of Embryo(s) Collected

When flushing the embryos from the donor is complete, a 30 minute wait period allows the embryos to settle on the filter. After the 30 minutes is complete the embryos flushed out can be found by a stereoscopic microscope. When an embryo is found it is washed and transferred to new fluid containing bovine serum.

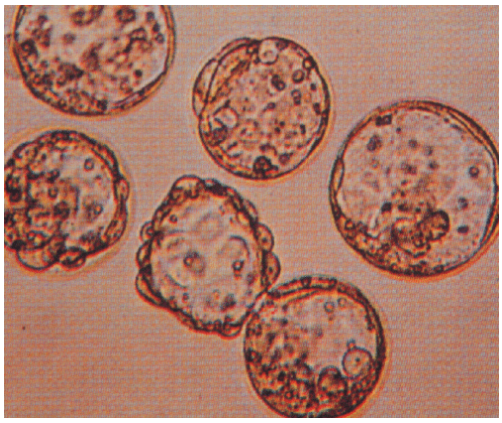


Figure 5: Cattle Embryos at Various Stages of Development (Morris, D.G. et all. 2001)

Table 1: Embryo Grading (Seidel, G.E., 1991)

Classification	Avg. %	Description
Excellent or Good	54%	Perfect or consisting of trivial imperfections. May be slightly asymmetrical or small.
Fair	30%	Definite but not sever abnormalities. Small size, or small amount of degeneration
Poor	8%	Considerable degeneration, vesculated cells, varying cell size, retarded by up to 2 days in development
Dead or Degenerate	8%	Severely degenerate; not worth transferring.

Once the embryos are located under the microscope, they are evaluated for stage of development and quality. The major criteria for evaluation consist of shape, size, color and texture of the cytoplasm, diameter, regularity of zona pellucida, presence of vesicles, and in some instances gender. Embryos classify numerically, meaning the greater potential embryo has to survive in the recipient the higher the numerical grade. These grades are referred to in Table 1.

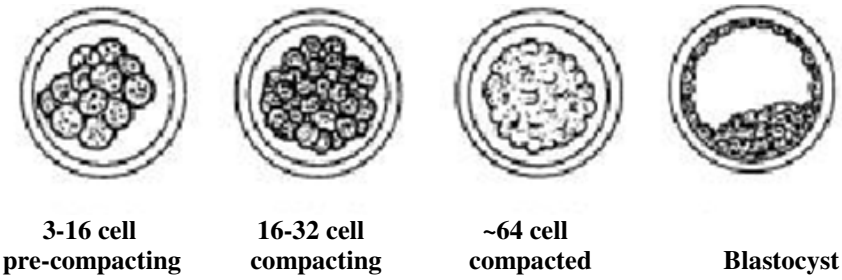


Figure 6: Embryo Development (Morris, D.G., et all. 2001)

In addition to being evaluated for quality, they are observed for their stage of development. This grade is done without consideration of quality. Table 2 refers to the stage of embryos in days after Estrus.

Table 2: Stages of Normal Embryonic Development (Seidel, G.E., 1991)

Stage	Status	Days after Estrus
1	Unfertilized	0-2
2	2-12 Cells	1-5
3	Early Morula	5-6
4	Morula	5-7
5	Early blastocyst	7-8
6	Blastocyst	7-9
7	Expanded blastocyst	8-10
8	Hatched blastocyst	9-11
9	Expanding hatched blastocyst	11-12

The observation is also made if the embryo is fertilized or not. You can get 20 embryos from a single donor but only 10 of them might be fertile. Healthy embryos can be transferred to a recipient that is one day, give or take, from the estrus of the donor. Or it can be froze and saved for a later date. (Kunkel, J.R., 2010)

Transfer into recipient

Since most embryos are received non-surgical and flushed from the uterine horn, most transfers are non surgical and transferred to the new recipient in the uterine horn. It is also much easier to transfer embryos into the uterus than the oviduct. While both surgically and non-surgically work well, non-surgical transfer can be done faster. (Seidel, G.E., 1991)

Surgically vs non-surgically transfer

Surgical transfers have been done two ways, via mid-line abdominal or flank. Incisions in the flank have been found much more practical, being set much closer to the

ovaries. When a flank incision is made the recipient is in a squeeze chute that allows a window for the flank to be cut. The flank is the area right above the fore udder (or where fore udder would be) near the stifle. The corpus lutem (CL) is located by rectal palpation and the flank in association to the side the CL is washed with soap and water then sterilized with iodine and alcohol. After being scrubbed, the surgeon will make a skin incision about 15 cm long, high on the flank, just anterior to the hip.

Figure 7: Incision Location

The muscle layers are separate and the peritoneum is cut. The surgeon inserts his hand and forearm to locate the ovary, usually 25 centimeters postier to the incision. Once the ovary has been located the surgeon then palpates the CL. The surgeon will grasp and stretch board ligament of the uterine horn with his/her thumb and forefinger. This is done very carefully knowing the uterine horn is very fragile. On the wall of the cranial third of the exposed horn a puncture wound is made with a blunted needle. An assistant nearby show draw up the embryo from the storage container with a small glass pipette (<1.5, outside diameter) with 0.1 ml of medium already inside. The pipette inserts into the lumen of the uterus and the embryo expels. The incision is closed using two layers of

sutures Although, surgical transfer only takes about 15 minutes it takes lots of experience and confidence that the embryo has been deposited in the lumen.

Non-surgical transfer is used more routinely but takes more patience and accuracy. A more accurate palpation of the ovaries needs to be made to ensure which side has ovulated. After determining if the recipient is able to accept embryos, the embryo transfer device is passed through the cervix. This process is slightly more difficult because of the luteal phase apposed to the oestrus phase when artificial insemination occurs and the cervix is more open. Heifers are a bigger challenge because they have a smaller cervix but have a much high conception rate than a larger cervix in an older cow. The next step is to insert the instrument into the desired uterine horn quickly and smoothly. Again a more difficult step to master but easier once artificial insemination is learned and practice is required.

Storage/ Freezing Embryos.

Embryos can be transferred right after collection or stored frozen to transfer at a later date. If embryos are going to be frozen it should be done within 3 to 4 hours of collection and froze and stored in liquid nitrogen. The embryo(s) should be placed in a saline-glycerol solution in preparation of freezing. Small straws, 0.25 or 0.5 cc straws are rinsed of toxic residues and filled half-way with a freezing medium, then an air bubble of 4-6mm in size, then more freezing medium that contains the embryo, leaving the straw 90 percent full. The straw is then sealed off with heat or polyvinyl chloride powder. Since the freezing and thawing process is very complex it usually results in an approximate 20 percent decrease in pregnancy rates that those that are freshly implanted. These next steps are done carefully and accurately to ensure a greater decrease of pregnancy rates does not occur. The straw is placed horizontally in the freezing machine and cooled to -7 degrees

Celsius. The temperature drops at -0.5 degrees Celsius per minute until -30 degrees Celsius is reached. Once -30 degrees Celsius is reached the straws are plunged into the liquid nitrogen for storage. Figure 8 illustrates the proper procedure that is explained above to place an embryo in a straw.

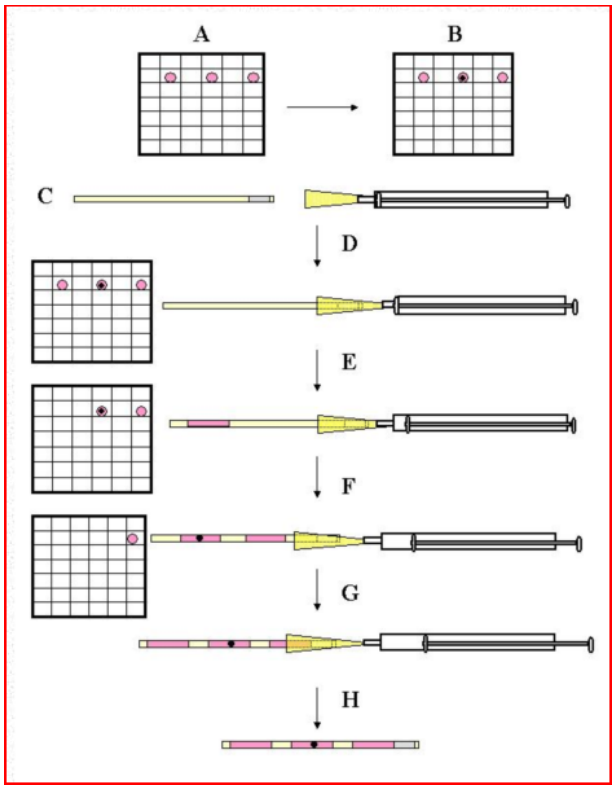


Figure 8: Embryo into a straw

Thawing is just as important as freezing. The embryo filled straw is held in the air for 20 seconds, followed by a 20 second bath in 37 degree Celsius water bath. Frozen embryos are a marketable commodity and have especially been useful in international sales of US genetics. Prices will be discussed in further sections.

Management Tool.

Embryo Recovery and transfers are now starting to become a very important management tool in the dairy industry. A simple example is with Cruachan Highland Cattle (Cruachan, 2010). E.T. technicians have produced up to ten or more progeny per year from their best cows with profits from the increased sale of quality genetics without losing the bloodlines. This allows an extended the family tree of older cows who are incapable of carrying another calf on their own, using the embryo transfer method. Conserve the genetics in their herd through the uses of, embryo freezing for, export, domestic sale or future transfers on their own farm. Introduce top genetics into the farmers herd, rapidly and economically from Australian and overseas. (Cruachan, 1992)

DNA

Parentage testing is done upon request. When calves are born and are being sold, buyers may ask for a DNA test to ensure they are getting the correct animal. First, be sure to enclose the cattle that are being tested in a close proximity. To gather the hair follicles tail or ear hairs are preferred. Forms can be sent from the Holstein Association directly to the site where the DNA collection will take place. A set of 20 hairs or more are needed for a thorough test that will be sent back to the Holstein Association. Further analysis will take place in their lab and results will be sent by mail. Figure 9, a few pages further, illustrates the proper collection and sending process for all DNA samples. Procedures are similar, but vary slightly for the U.S. Jersey Association.

METHODS AND MATERIALS

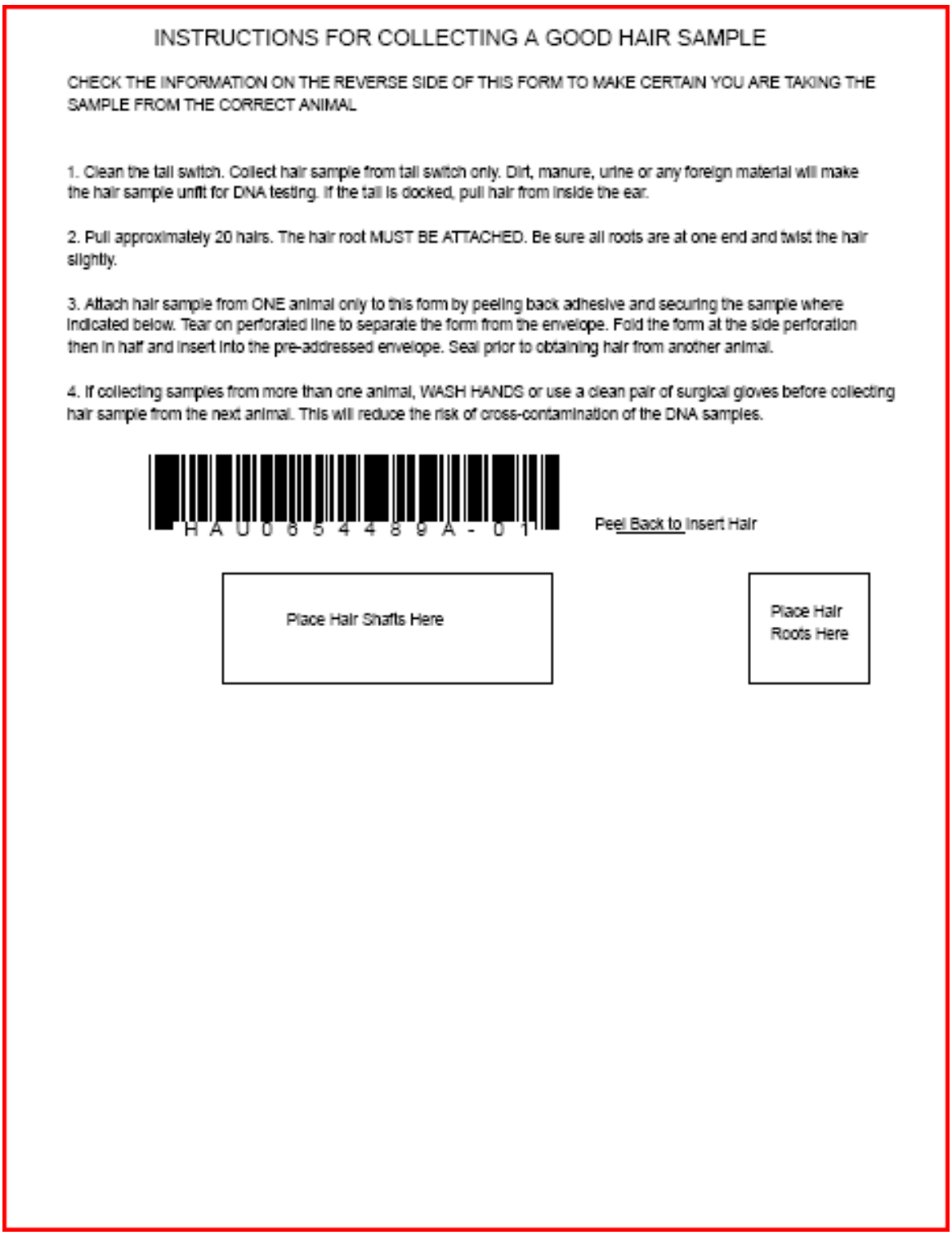
During the Literature Review I went over some of the basic methods and benefits of Embryo Recovery and Transfer. With great help from Dr. Stan Henderson, Mr. Rich Silacci and Ms. Daniel Demertrio the Cal Poly embryo data was collected and analyzed to determine the current number of frozen embryos, live ET calves and current living donors. The Cal Poly success rates will be compared to those of RuAnn Dairy with frozen and total embryo numbers. The Embryo Transfer information further ahead with help prove the efficiency and future value to the Cal Poly herd.

First, a deep understanding of previous records and record systems was analyzed. Ms. Demertrio keeps accurate records of all animals kept and have had or having embryo work done at Ruann. Her current record system is understandable mostly and the system created for the Cal Poly dairy will mimic her idea and pattern. Although similar, they differentiate in that the Cal Poly system will break down each individual donor and recipient to keep exact records of embryo, fertilization to birth. The idea to keep the two documents similar is explained by allowing the records to be combined easily for Cal Poly animals not housed in San Luis Obispo. This will be more concise and less confusing for up keeping.

An excel format will be used to create charts, tables and lists. This will allow easy changes to be made to prior spreadsheets and to make new summary sheets as new animals enter the program. A hard drive and a hard copy with be obtained by the herd manager at all times, for available access at all times. The hard copy will consist of a binder with print out sheets from the excel file. Each individual donor will be sectioned out by tabs in numeric order. Within each section for the donor there will be multiple

documents pertaining to breed registration, pedigree (optional), flush records, implant certificates, frozen embryos records as well as the individual sheet created from the excel file(all copies of orginial). A current list of contacts, frozen embryos, implant recipients and an overview of proceeding data will be stored in the first few pages of the first tab within the binder. Having this information all together in one place gives a quick and effortless search on an embryo that may be of interest of a sale as well as a pedigree and ensures an official procedure of collection and registration was complete.

Noted earlier, DNA sampling may be requested. Below in figure 9 is an example of a DNA collection form. At least 20 hairs must be pulled from the switch (long hairs on the tail). The switch should be clean of manure, dirt or any foreign material. To be effective the hairs must have the roots attached and all facing in the same direction. To ensure at least 20 hairs with roots are pulled, pliers are useful for a secure grip. Figure 9 shows two large boxes. The longer more rectangle shape is the hair end while the short more square shape is where the roots are placed. Between the two is a reseal able tape that keeps the hairs grouped together and stays with the correct form. When doing multiple collections at once, it is advised to wash hands or change gloved to reduce the risk of cross contamination. The results section will show how a returned document appears, after being sent back from the appropriate breed association. The two examples used here are from the Holstein Association.



INSTRUCTIONS FOR COLLECTING A GOOD HAIR SAMPLE

CHECK THE INFORMATION ON THE REVERSE SIDE OF THIS FORM TO MAKE CERTAIN YOU ARE TAKING THE SAMPLE FROM THE CORRECT ANIMAL

1. Clean the tail switch. Collect hair sample from tail switch only. Dirt, manure, urine or any foreign material will make the hair sample unfit for DNA testing. If the tail is docked, pull hair from inside the ear.

2. Pull approximately 20 hairs. The hair root MUST BE ATTACHED. Be sure all roots are at one end and twist the hair slightly.

3. Attach hair sample from ONE animal only to this form by peeling back adhesive and securing the sample where indicated below. Tear on perforated line to separate the form from the envelope. Fold the form at the side perforation then in half and insert into the pre-addressed envelope. Seal prior to obtaining hair from another animal.

4. If collecting samples from more than one animal, WASH HANDS or use a clean pair of surgical gloves before collecting hair sample from the next animal. This will reduce the risk of cross-contamination of the DNA samples.

HAU065448 9A-01

Peel Back to Insert Hair

Place Hair Shafts Here

Place Hair Roots Here

Figure 9: DNA Sample Collection Work (Holstein Association, 2009)

Efficiency and Economics

An important aspect to be aware of before starting any new program is the effectiveness and viability of that program. An E.T. program is no different when it comes to measuring the cost and efficiency. After working close with Ms. Daniella Demetrio and Dr. Stan Henderson, embryo recovery is complied on a monthly basis over the previous three years for the Ruann & Maddox Dairy. The following table illustrates that on

Flush Program 2007-2009			
	Total	Dry Cows	Milk Cows
Number of Super Stimulated Cows	1944	986	1008
Number of Flushed cows	1811	953	858
Percent	93%	97%	85%
Total Structures	20041	12963	7078
Structures Per Cow	11.1	13.6	8.2
Viable	9382	5440	3942
Viable per Cow	5.2	5.7	4.6
Percent	47%	42%	56%
Fresh	3266	1937	1329
Frozen	6116	35.3	2613
Inviable	10659	7523	3136
Percent	53%	58%	44%
Unfertile	9500	6608	2892
Degenerate	1159	915	244

Table 3: Ruann & Maddox Dairy Embryos 2007-09

average 50% of the viable embryos were viable. Lactating cows were 14% more viable than dry cows showing an average of 47%. The total percent of numbered of cows flushed when compared to number of stimulated cows was 93% with 47% in possible transfers. The average number of viable embryos per cow is 5.2. The dry cows used in the program were continuously used in the embryo transfer program which may impact the lower percentage of viable embryos.

After reviewing Ruann & Maddox a Cal Poly list was generated for comparison. The table following displays fresh and frozen embryos with percent pregnancy in heifers. When compared to Ruann to Maddox total percentage 67.1% and 59.2%, respectively, the average being 61.2% or 1130 cows.

ET-Heifers 2007-2009 Conception Rate				
		Total	Ruann	Maddox
	Total	2911	802	2109
Fresh	Preg	1832	555	1277
	Percent	62.93%	69.20%	60.00%
	Total	910	166	744
Frozen	Preg	508	95	413
	Percent	55.80%	57.20%	55.50%
	Total	3821	968	2853
Total	Preg	2340	650	1690
	Percent	61.20%	67.10%	59.20%

Table 4: Ruann & Maddox Conception Rate

Consideration is taken in account for the 7% difference between the fresh and frozen conception rates. This is a big difference to the national average, which has a tendency to very as much as 20% between fresh and frozen implant conception rates.

Cost

The cost of embryo transfer has many different variables. Some of which include where the flush and transfer will be taking place, if sexed semen will be used and whether surgical or non-surgical procedures are used. The location where the flush and or transfer takes place is a big factor. If done with an embryo transfer companies on their facility, the procedure is done in hospital similar conditions. Although, most flushes and transfers take place on farm. Most technicians are equipped with the knowledge to freeze and store embryos that are not planned to be freshly implanted. The average flush cost at Ruann is $460 per Holstein cow. Using the average of number of viable embryos per cow of 5.2, the average cost per embryo is $88.57. The chart following was provided by Ruann Dairy showing average cost of materials, hormones, fees and labor.

Table 5: Cost analysis of Embryo Transfer

	Unit	Cost/Unit	Total Cost
Superovulation and AI			
Pluset	1.2	$110.00	$132.00
Prostaglandin	4	$2.20	$8.80
GnRH	1	$2.30	$2.30
Others	1	$5.00	$5.00
Semen	2.5	$25.00	$62.50
			$210.00
Flushing			
Ringer Solution	1.8	$2.00	$3.60
Flushing Media (PBS) 2 liters	0.0625	$31.00	$1.94
Holding Solution	0.17	$18.00	$3.00
Ethilene Glycol	0.2	$14.00	$2.80
Filter	1	$8.10	$8.10
Catherter/Silicone Tube	1	$4.00	$4.00
6-well Dish	1	$2.95	$2.95
100x20 dish	1	$0.50	$0.50
Straws 0.25cc	5.2	$0.22	$0.22
Straws 0.50cc	2.6	$0.85	$4.42
Tips	1	$0.10	$0.26
Syringes 12cc	1	$0.35	$0.35
Syringes 20cc	1	$0.44	$0.44
Racks, goblets, tabs…	1	$1.00	$1.00
Others (pen, needle, pipette, etc)	1	$5.00	$5.00
			$38.93
Other Costs			
Food (each donor 2 months)	60	$2.00	$120.00
Holstein Association	1	$5.00	$5.00
AI and Super labor	1	$15.00	$15.00
Flush Labor	1	$30.00	$30.00
Lab Labor	1	$15.00	$15.00
			$185.00
Total			
Superovulation and AI	1.1236	$210.60	$236.36
Flushing	1	$38.93	$38.93
Other Costs	1	$185.00	$185.00
Total	**5.2**		**$460.56**
Total per Embryo	**1**	**$88.57**	

Table 5 on the previous page gives a detailed breakdown of expenses for an average embryo transfer. The national average of viable embryos per embryo collection

is 5.2. After calculating the Cal Poly Dairy's average of viable embryos it difference was almost double. Cal Poly dairy's average viable embryos per collection are 10.9. When the total cost of $460.56 is divided with the Cal Poly viable average of 10.9 instead of the national average 5.2, the cost total per embryo is $42.25. Cal Poly currently pays $50.00 an embryo. Keep in mind that the prices stated in table 5 are national averages and labor costs may change company to state.

Stated before, embryo transfer can take place on the E.T.'s facilities of the company that is being worked with. Some of these facilities may provide recipient(s) for your embryo(s) in addition to their services. This would increase the cost with an additional fee of $1,200 to $ 1,800 per pregnancy. Many purebred operations conducting embryo transfer on a regular basis consider that each "ET" calf must have a market value of $1,500 to $2,000 greater than other naturally conceived and reared calves in the herd before embryo transfer is considered (Mitchell, J.R., et al, 2004). Producers, who decide to do E.T. work with their animals, should contact the appropriate breed association to ensure proper registration and certification is in place for each embryo transfer animal that is born.

RESULTS AND DISCUSSION

Embryo Collections of Cal Poly Cows

Cal Poly currently has a total of 14 cows in their flush program. By natural causes and new cows arising these numbers are subject to change, in both positive and negative directions. With these 14 cows, Cal Poly has a total of 295 embryos either freshly implanted or froze within the past 8 months. Table 6 shows this information in a quick and easy readable format. This table includes the date the flushing took place, the donor

Table 6: Collections of Cal Poly Cows at Ruann

Date	Cow	Sire	Fresh	Frozen	VIABLE	TOTAL	PS
3/2/2010	**889**	200J430	3	1	4	4	Fresh embryos transferred at Golden Genes.
4/29/2010	**889**	7JE867	0	3	3	15	
5/7/2010	**1940**	29H12209	0	1	1	9	
6/14/2010	**1533**	7H8190	0	9	9	14	
6/14/2010	**2127**	11H8342	0	8	8	26	
6/22/2010	**643**	7JE1000	2	6	8	13	Fresh embryos transferred at Maddox Dairy.
6/28/2010	**889**	200JE303	2	14	16	16	Fresh embryos transferred at Maddox Dairy.
7/30/2010	**1945**	200H3205	0	7	7	33	
8/5/2010	**1940**	7H8190	0	6	6	12	
8/17/2010	**741**	29JE3506	0	11	11	16	
8/18/2010	**2127**	7H10506	0	17	17	21	
8/20/2010	**740**	200J303	3	15	18	25	Fresh embryos transferred at Maddox Dairy.
8/31/2010	**889**	29JE3506	0	0	0	2	
8/31/2010	**703**	29JE3506	0	0	0	10	
9/13/2010	**643**	29JE3506	0	3	3	12	
10/14/2010	**740**	200JE303	1	0	1	16	Fresh embryo transferred at Maddox Dairy.
10/15/2010	**1945**	200H3205	0	1	1	31	
10/27/2010	**741**	29JE3506	0	14	14	16	
11/1/2010	**703**	29JE3506	0	1	1	20	
Total:					208	311	

Percent Viable: 67%

cow's identification number, the sire of the embryos, how many were implanted fresh and number that were frozen, total viable embryos, the total embryos collected and important side notes. This table gives an overall view of each flush and the results that is easily assessable and understandable.

Inventory of frozen embryos stored at Ruann

A total of 173 of the 295 embryos were frozen and are being kept for later use, whether it be for sale or for the use of Cal Poly dairy. Most of these embryos are

Table 7: Inventory of Frozen Holstein and Jersey Embryos Stored at Ruann

HOLSTEIN

TAG	NAME	NAME	CANE	QUANT	SALE
1533	BEST-SUNRISE BLITZ JW DARCI	GEN-MARK STMATIC SANCHEZ	9119	8	8
1533	BEST-SUNRISE BLITZ JW DARCI	BRAEDALE GOLDWYN	9181	8	8
1940	POLY LEE BEAUTY	GEN-MARK STMATIC SANCHEZ	9147	6	6
1945	POLY DURHAM JULIE	BRAEDALE GOLDWYN	9145	7	4
1945	POLY DURHAM JULIE	BRAEDALE GOLDWYN	9210	1	1
2127	POLY HI METRO BARBIE	REGANCREST HHF MAC ET	9120	6	6
2127	POLY HI METRO BARBIE	MAPLE DOWNS IGW ATWOOD ET	9168	9	9
2127	POLY HI METRO BARBIE	MAPLE DOWNS IGW ATWOOD ET	9169	8	6
				53	48

JERSEY

TAG	NAME	NAME	CANE	QUANT	SALE
106	POLY HALLMARK JOELLE	MAACKDAIRY REGION ET	9073	8	8
179	POLY JADE ALLIE	MAACKDAIRY REGION ET	9067	8	8
179	POLY JADE ALLIE	MAACKDAIRY REGION ET	9068	10	10
179	POLY JADE ALLIE	RICHIES JACE T-BONE A364	9072	2	2
179	POLY JADE ALLIE	SHF CENTURION SULTAN	9086	9	9
643	POLY GOLDEN BOY JUNIPER TW	SC GOLD DUST PARAMOUNT IATOLA	9066	7	7
643	POLY GOLDEN BOY JUNIPER TW	SC GOLD DUST PARAMOUNT IATOLA	9074	2	2
643	POLY GOLDEN BOY JUNIPER TW	RICHIES JACE T-BONE A364	9087	5	5
643	POLY GOLDEN BOY JUNIPER TW	RICHIES JACE T-BONE A364	9133	6	6
643	POLY GOLDEN BOY JUNIPER TW	TOLENAARS IMPULS LEGAL 233 ET	9189	3	3
703	POLY HALLMARK HOLLETTE	TOLENAARS IMPULS LEGAL 233 ET	9218	1	1
740	POLY HALLMARK HALLIE	SHF CENTURION SULTAN	9171	10	10
740	POLY HALLMARK HALLIE	SHF CENTURION SULTAN	9172	5	5
741	POLY PARAMOUNT OPAL	TOLENAARS IMPULS LEGAL 233 ET	9160	7	7
741	POLY PARAMOUNT OPAL	TOLENAARS IMPULS LEGAL 233 ET	9161	4	4
741	POLY PARAMOUNT OPAL	TOLENAARS IMPULS LEGAL 233 ET	9216	9	9
741	POLY PARAMOUNT OPAL	TOLENAARS IMPULS LEGAL 233 ET	9217	4	4
807	POLY JADE JADE	SHF CENTURION SULTAN	9088	3	3
889	POLY JACE HALLIE	CHASIN-RAINBOWS ACT RILEY ET	9063	5	5
889	POLY JACE HALLIE	GRIFFENS GOVERNOR ET	9098	3	3
889	POLY JACE HALLIE	SHF CENTURION SULTAN	9135	10	10
889	POLY JACE HALLIE	SHF CENTURION SULTAN	9136	4	4
					125

considered high in value; this is due to the fact of the genetics in the parentage of the embryo. Some of these embryos have already been marketed and sold during the Cal Poly Classic Sale that occurred on October 15, 2010. Further involvement with the sale will be explained later.

Above table 7 shows the inventory of both Holstein and Jersey frozen embryos on file as of November 11, 2010. These tables are set up similar to table 6 to continue with an understandable format. The first column is the donor cow identification number,

followed by registration name, sire's full name, cane number where the embryos are stored and the number of embryos stored in each cane. The sale column is referring to the number of frozen embryos that were sold at Cal Poly Classic Sale and still available for purchasing.

Individual cow data

Largely the data is shown in an overview of all donor cows and recipients together. A table was created to pull out specific information for each individual donor and recipient.

Table 8: Individual donor sheet

740

# Frozen	Fresh Im.	Total Eggs	Sire	STW #	Flush Date	Cane
15	3	25	Sultan		8/20/2010	9171/9172

from cane 9171 sold 10 to Kisst @ 250 (CPCS)

Recipients

Sire	Recip. #	Imlant Date	E. Quality	STW #	P/O	Due Date	M/F
Sultan	83393	8/20/2010	1	1			
Sultan	82557	8/20/2010	1	2	preg	5/22/2011	
Sultan	83157	8/20/2010	1	3	preg	5/22/2011	

Table 8 shows an example of a single donor's individual chart. The donor cows number is stated center bold with emphasis so not to get confused which individual is being evaluated. The top chart explains the number of frozen, fresh and total embryos that were extracted on the flush date provided. It also shows the straw number the embryos are being held if froze. There is also a side note added to this individual's

information because 10 of her embryos were sold at a price of $250 each to the Kisst Dairy. The bottom table refers to the recipients of the donor cow's embryos. Starting with the sire information to help refer back to which flush date it may be referred to. Followed by the recipient identification number, date embryos were implanted, embryo quality, straw number, whether the pregnancy kept or she was found open, the recipients due date and finally whether the embryo was male or female. This chart takes the reader from start to finish of the embryo course, flush to birth. This table has been created for each donor cow and has been kept up to insure accurate and most current information is held at all times.

Pregnant recipients

To complete the quick overview, a table of just pregnant recipients is obtained in addition to the tables that have already been discussed. Table 9 shows the date the embryo was implanted, donor cow's identification number, sire of the embryo, recipients identification number, location of the recipient, the diagnoses of pregnancy, the recipients due date as well as the sex and control number of the calf. This chart allows for these recipients to get paid slightly more attention when it comes to calving time, allowing proper notes and paper work to be filed.

Table 9: Pregnant Recipients

DATE	DONOR	SIRE	RECIP	LOCATION	DIAGNOSTICS	DUE	CALF
03/02/10	1945	29H12209	**85166**	Ggenes	Pregnant	2-Dec-10	
03/02/10	1945	29H12209	**96083**	Ggenes	Pregnant	2-Dec-10	
03/02/10	1945	29H12209	**96110**	Ggenes	Pregnant	2-Dec-10	
03/03/10	889	200JE430	**85127**	Ggenes	Pregnant	3-Dec-10	
03/03/10	889	200JE430	**96101**	Ggenes	Pregnant	3-Dec-10	
03/05/10	1904	29H12209	**96104**	Ggenes	Pregnant	5-Dec-10	
03/05/10	1904	29H12209	**87784**	Ggenes	Pregnant	5-Dec-10	
03/05/10	1904	29H12209	**94045**	Ggenes	Pregnant	5-Dec-10	
03/05/10	1904	29H12209	**94090**	Ggenes	Pregnant	5-Dec-10	
03/05/10	2126	7H5157	**94076**	Ggenes	Pregnant	5-Dec-10	
03/05/10	2126	7H5157	**96109**	Ggenes	Pregnant	5-Dec-10	
03/05/10	2126	7H5157	**96087**	Ggenes	Pregnant	5-Dec-10	
03/05/10	2126	7H5157	**96107**	Ggenes	Pregnant	5-Dec-10	
03/05/10	2126	7H5157	**94006**	Ggenes	Pregnant	5-Dec-10	
08/20/10	740	200JE303	**82557**	Maddox	Pregnant	22-May-11	
08/20/10	740	200JE303	**83157**	Maddox	Pregnant	22-May-11	

Cal Poly Classic Sale

The Cal Poly Classic Sale was held Friday, October 15, 2010 in the pavilion on the Cal Poly dairy. The pavilion was packed full of supporters, Cal Poly alumni, professors and current Cal Poly students. There were 70 lots total, 55 Holstein and 15

Table 10: Sale paperwork to collect

Holsteins

1. Nothing
2. Nothing
3. Cal Poly-Bill of sale, reg
4. Complete
5. Nothing
6. Bill of Sale
7. Nothing
8. Bill of Sale, Registration
9. Nothing
10. Nothing
11. Bill of Sale, registration
12. Nothing
13. Nothing
14. Nothing
15. Nothing
16. Bill of Sale
17. Complete
18. Nothing
19. Bill of Sale
20. Complete
21. Nothing
22. nothing
37. Nothing
38. Nothing
39. Cal poly-embryos
40. Nothing
41. complete
42. Complete
43. Complete
44. Bill of Sale
45. TB
46. Bill of Sale and TB
47. Nothing
48. Nothing
49. Complete
50. Nothing
51. TB
52. Complete
53. Cal Poly- Bill of Sale
54. Nothing
55. Nothing

Jerseys

1. Nothing
2. Registration

Jersey ranging from live animals to embryos. The Holstein live lots averaged $3,024 and embryos averaged $438. The Jerseys averaged $2,732 live and $312.50 for embryos. The previous tables and charts helped determine how many embryos were viable to sell, having both quality and quantity. Cal Poly sold 30 embryos in the Cal Poly Classic Sale, and an additional 50 are being marketed through World Wide Sires. By having a total of almost 200 embryos, it was very useful to utilize these tables in order to make the decision of which embryos to sell. The sale was very profitable to the dairy and was kept organized with the help and support from the sale committee. Table 10 is a list and was provided to the committee to help get the proper sale paperwork in order and sent to the appropriate buyers. Table 10 shows a snip of each lot and the correct paperwork that was

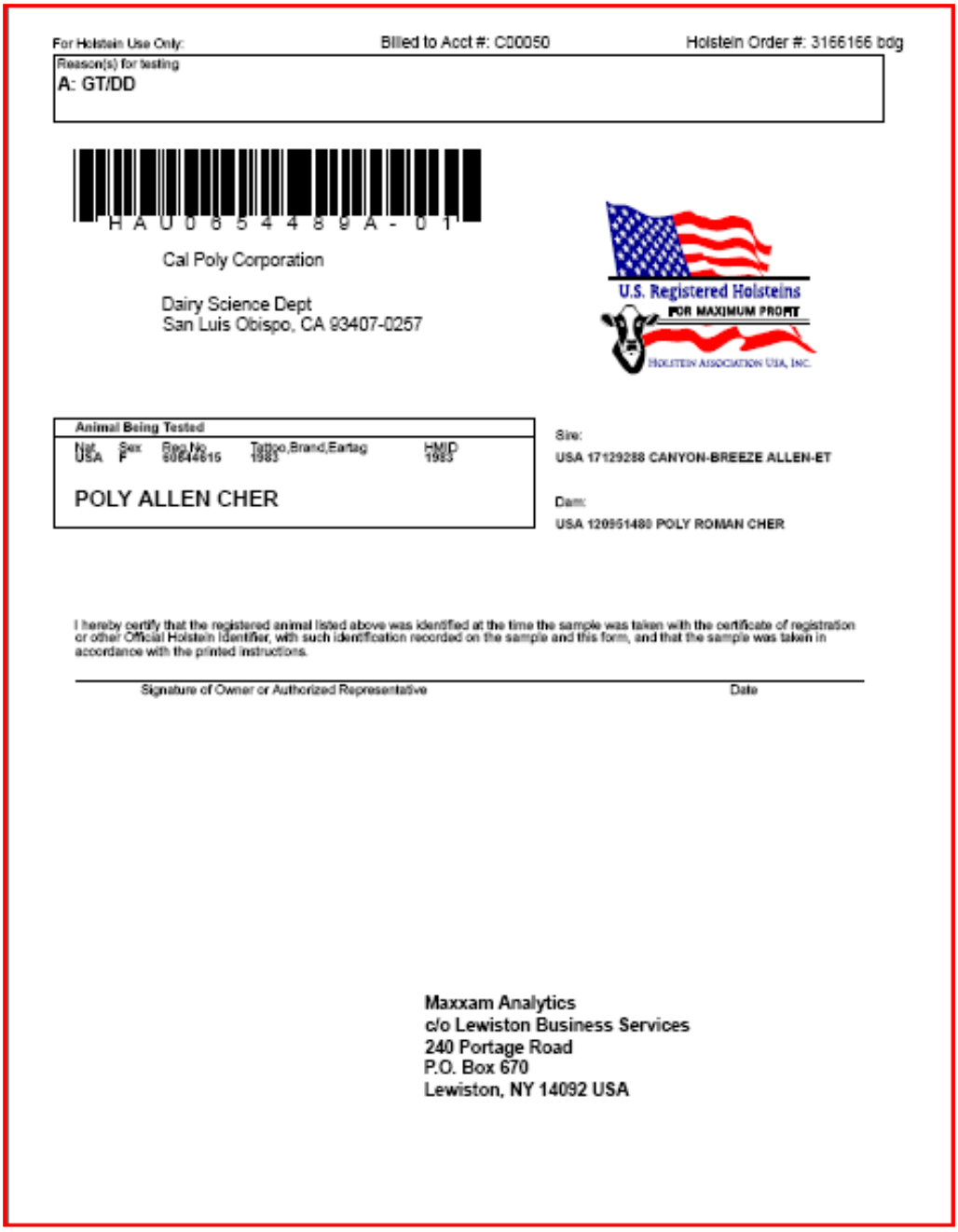
provided before the sale and what was left to collect from original owners. An excel sheet was also created to provide sold embryos from the sale with the price at which they were sold and the potential embryos to be sold, table 11.

Table 11: Sold and Potential to Sell Embryos

	Embryos that could be offered:						
HOSTEIN	1533	BEST-SUNRISE BLITZ JW DARCI	GEN-MARK STMATIC SANCHEZ		9119	3	*Vierra Dy @325*
HOSTEIN	1533	BEST-SUNRISE BLITZ JW DARCI	BRAEDALE GOLDWYN		9181	8	
JERSEY	179	POLY JADE ALLIE	MAACKDAIRY REGION ET		9067	3	
JERSEY	179	POLY JADE ALLIE	MAACKDAIRY REGION ET		9068	10	*Kist @ 325*
JERSEY	179	POLY JADE ALLIE	RICHIES JACE T-BONE A364		9072	2	
JERSEY	179	POLY JADE ALLIE	SHF CENTURION SULTAN		9086	9	*Kisst @ 325*
JERSEY	889	POLY JACE HALLIE	SHF CENTURION SULTAN		9135	5	*Kisst @250*
JERSEY	889	POLY JACE HALLIE	CHASIN-RAINBOWS ACT RILEY ET		9063	5	
JERSEY	889	POLY JACE HALLIE	GRIFFENS GOVERNOR ET		9098	3	
JERSEY	889	POLY JACE HALLIE	SHF CENTURION SULTAN		9136	4	
JERSEY	740	POLY HALLMARK HALLIE	SHF CENTURION SULTAN		9171	10	*Kisst @ 250*

DNA

Materials and methods explained the process of collection DNA if a parentage test was asked for. Below in figure 10 is what follows the DNA testing at the lab. The barcode on both figure 9 and 10 are the same. Each individual animal has their own bar code with all of their information stored in it. After retaining the DNA sampling information back from the appropriate breed association, records can be kept that a parentage test was completed and calves to be born from her transfer embryos can be confirmed hers, after a DNA test of that offspring. This document clearly confirms animals full name, sex, identification number, sire and dam, along with the owner's name.



For Holstein Use Only: Billed to Acct #: C00050 Holstein Order #: 3166166 bdg

Reason(s) for testing:
A: GT/DD

HAU0654489A-01

Cal Poly Corporation

Dairy Science Dept
San Luis Obispo, CA 93407-0257

U.S. Registered Holsteins
FOR MAXIMUM PROFIT
HOLSTEIN ASSOCIATION USA, INC.

Animal Being Tested

Nat	Sex	Reg No	Tattoo,Brand,Eartag	HMD
USA	F	60644815	1983	1983

POLY ALLEN CHER

Sire:
USA 17129288 CANYON-BREEZE ALLEN-ET

Dam:
USA 120951480 POLY ROMAN CHER

I hereby certify that the registered animal listed above was identified at the time the sample was taken with the certificate of registration or other Official Holstein Identifier, with such identification recorded on the sample and this form, and that the sample was taken in accordance with the printed instructions.

Signature of Owner or Authorized Representative Date

Maxxam Analytics
c/o Lewiston Business Services
240 Portage Road
P.O. Box 670
Lewiston, NY 14092 USA

Figure 10: DNA Sample Collection Work (Holstein Association, 2009)

CONCLUSION

In conclusion, a product was produced to enhance the accessibility of an effective embryo transfer record system to restore confusion of high student employee turn over rates. This product included a filing system with overall and individual data that can be easily kept up with excel worksheets. With great help from Dr. Stan Henderson, Mr. Rich Silacci and Ms. Daniela Demertio, this system should up hold and create less confusion between methods of data entry and help keep all records current and organized. Recipients will be easier to track and will be watched closer with more attention to details. Organization and efficiency are important to reduce confusion in any work environment. With more embryos to be collected and sales to be made, Cal Poly's turnover rate should shortly impact the income of the dairy to become more profitable and worldwide admired. Although successful pregnancy rates are higher at Ruann and Maddox, Cal Poly does have a higher viability percent of embryos. Cal Poly dairy's viable embryos are 10.9 per collection which is doubled the national average and viable percent to total embryos collected of 67%. With high viability rates Cal Poly has the right tools to continue to build the program and allow World Wide Sires to market their embryos for national use. The continued use of this simple recording system at the Cal Poly Dairy will increase the system's accuracy and efficiency, therefore ensuring a prosperous and successful future in the industry.

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